IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: **Bennett and Dobie**

Serial No.: **10/020,478** Group Art Unit: **1635**

Filed: **December 13, 2001** Examiner: **Jane J Zara**

Title: **Antisense Modulation Of B-Cell Associated Protein Expression**

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

DECLARATION UNDER 37 CFR §1.132

I, Dr. Susan Freier, a citizen of the United States, residing at 2946 Renault Street, San Diego, CA 92122, state and declare as follows:

1. I hold a B.A. in Mathematics (1972) from Carleton College, Northfield, Minnesota and a Ph.D. in Chemistry (1976) from the University of California, Berkeley, California.

2. I have been employed by Isis Pharmaceuticals, Inc. ("Isis"), for about fourteen years. Isis, the assignee of the above-identified patent application, specializes in oligonucleotide technology and uses the latest in bioinformatics programs to identify sites on selected genes for oligonucleotide screening. I am presently the Executive Director of antisense lead identification at Isis and am responsible for several projects. I lead a project utilizing antisense oligonucleotides for functional genomics of novel targets, including the use of computational genomics to characterize target RNAs and their variants, rapid throughput screening to identify active antisense oligonucleotides for novel targets, and Q-RT-PCR and microarrays for expression analysis. I also lead a project for determining microRNA function in mammals, including the use of computational identification of microRNAs and microRNA targets and the use of functional genomics to characterize microRNA biology and identify therapeutic applications of modulation

of miRNA activity. In addition, I lead a group charged with the identification and characterization of novel mechanisms for antisense oligonucleotides, including the use of computational genomics to identify mRNA variants, alteration of RNA processing, evaluation of siRNA and miRNA mechanisms. Another project I am responsible for involves biophysical and biochemical evaluation of novel antisense oligonucleotides, including the evaluation of thermodynamics and kinetics of hybridization to oligonucleotide and large structured targets, evaluation of the biochemical properties of novel oligonucleotides, characterization of antisense activity in cell assays, and protein-oligonucleotide binding.

3. As early as 1995, I performed and supervised experimentation employing oligomeric compounds and inhibition of mRNA expression. My work has involved designing assays to screen oligomeric compounds against specific genes as well as interpreting the results from such assays. I have authored or co-authored numerous scientific journal articles regarding the same. I am an expert in the art of antisense technology and oligonucleotide screening. A copy of my *curriculum vitae* is attached as Exhibit 1.

4. I have read the Office Action dated May 5, 2004 and understand that claims 1, 2, 4-16, and 21-26 of the above-identified application have been rejected as allegedly being obvious over the combination of the following references: Montano *et al.* Milner *et al.* (Nature Biotechnology, 15:537-541, hereinafter, the "Milner reference") and McKay (U.S. Patent No. 6,133,246 ,hereinafter, the "McKay reference"). I make this declaration to rebut the statements in the Office Action regarding the alleged reasonable expectation of success by one of skill in the art for inhibiting the expression of B-Cell Associated Protein.

5. It is not currently possible to predict the level of inhibition of expression achieved against a particular gene with any particular oligomeric compound prior to carrying out the appropriate experiments. It is also not reasonable to expect for any particular gene or mRNA that any number of oligomeric compounds exhibiting at least 42% inhibition of expression, as stated in claim 1 of the present application, will be obtained.

6. Each gene is unique. For instance, if one skilled in the art achieved at least 42% inhibition in the expression of a first gene with oligonucleotides that are targeted to the first gene, one skilled in the art ***would not*** reasonably expect success in achieving at least 42% inhibition in the expression of a ***different*** gene with a ***different*** set of oligomeric compounds that are targeted to the different gene or mRNA. The level of inhibition of expression that is observed for one target has no bearing on the level of inhibition of expression expected for a different target.

7. For example, as indicated by Exhibits 2 (inhibition of human tyrosine kinase, non-receptor, 1 mRNA expression in T-24 cells) and 3 (inhibition of rat urate anion exchanger 1 mRNA expression in Rin-M cells), 80 oligomeric compounds (each being 2'-O-methoxyethyl gapmers) were examined for their ability to inhibit expression (please note that the results are presented as % expression of the control). Referring to Exhibits 2 and 3, no oligomeric compounds were able to inhibit expression by at least 42%. It is **currently not** possible to predict **before** the appropriate experiment is performed, which targets will generate oligomeric compounds that will have a desired level of inhibition of expression.

8. This evidence demonstrates that one skilled in screening of oligomeric compounds cannot, *a priori*, reasonably expect a particular level of inhibition (i.e., such as at least 42%) of a gene or mRNA simply because methods of screening oligomeric compounds are available and/or routine. It is not acceptable to extrapolate results obtained for oligomeric compounds targeted to a particular gene to the results that would be reasonably expected for a different set of oligomeric compounds targeted to a different gene. Thus, the statements in the Office Action regarding reasonable expectation of success are neither accurate nor capable of being supported.

9. I am well aware that numerous U.S. Patents have been issued for oligomeric compounds that are able to inhibit the expression of a particular gene or mRNA. As stated above, however, the level of inhibition of expression that is observed for one particular target has no bearing on the level of inhibition of expression that can reasonably be expected for a different target. Indeed,

targets for which no inhibition or unsatisfactorily low inhibition of expression is achieved are rarely, if ever, the subject of a publication in a scientific journal, let alone are the subject of a patent application.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date:_____ By:_____

Susan Freier, Ph.D.

Attachments:

Exhibits 1, 2 and 3

CURRICULUM VITAE

Susan M. Freier
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Executive Director Antisense Lead Identification
ISIS Pharmaceuticals
2292 Faraday Avenue
Carlsbad, California 92008
(760) 603-2345
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EDUCATION:
University of California, Berkeley, California
Ph.D in Chemistry, 1976

Carleton College, Northfield, Minnesota
B.A. in Mathematics, summa cum laude, 1972

AWARDS:
Damon Runyon-Walter Winchell Cancer Fund Fellow (1976-1978)
California Regents Fellow (1974-1976)
NSF Graduate Trainee (1972-1973)

OTHER:
Served on Genome Study Section NIH 1997-2002, Chair 2000-2002

EXPERIENCE:

ISIS Pharmaceuticals San Diego 1990-present

Current title: *Executive Director Antisense Lead Identification*

- Determination of microRNA function in mammals. Includes computational identification of miRNAs and miRNA targets. Functional genomics to characterize miRNA biology and identify therapeutic applications of modulation of miRNA activity.
- Use of antisense oligonucleotides for functional genomics of novel targets. Includes: Computational genomics to characterize target RNAs and their variants, rapid throughput screening to identify active antisense oligonucleotides for novel targets, Q-RT-PCR and microarrays for expression analysis.
- Identification and characterization of novel mechanisms for antisense oligonucleotides. Includes computational genomics to identify mRNA variants, alteration of RNA processing, evaluation of siRNA and miRNA mechanisms.
- Biophysical and biochemical evaluation of novel antisense oligonucleotides. Includes: thermodynamics and kinetics of hybridization to oligonucleotide and large structured targets, evaluation of biochemical properties novel

oligonucleotides, characterization of antisense activity in cell assays, protein-oligonucleotide binding.

- Characterization and screening of combinatorial libraries. Includes: theoretical and experimental evaluation of strategies for deconvolution, high throughput screening of combinatorial libraries, bacterial RNA-protein interactions.

Molecular Biosystems Inc.
San Diego
1986-1990

- Development of non-radioactive DNA oligonucleotide probe based tests for detection of infectious and genetic diseases. Experience in: isolation of DNA from clinical samples, probe design, hybridization optimization, assay simplification, process validation. Includes: development of FDA cleared clinical tests for the direct detection of rotavirus or *Campylobacter* in stool, development of colony filter tests for bacterial identification and *in situ* hybridization tests for detection of virus in fixed tissues, cultured cells or patient specimens.

University of Rochester
Rochester NY
1979-1985

- Postdoctoral research with Douglas H. Turner on nucleic acid structure and dynamics. Experience in: chemical and enzymatic synthesis of oligonucleotides (deoxy- and ribo-), hybridization thermodynamics and kinetics, development of a laser temperature jump apparatus, NMR spectroscopy, computer programming and interfacing to laboratory instruments.

Northwestern University
Evanston IL
1976-1979

- Postdoctoral research with Irving M. Klotz and Richard P. Van Duyne on resonance Raman spectroscopy of DNA-mutagen interactions and resonance Raman spectroscopy of hemerythrin. Experience in: protein isolation, laser Raman spectroscopy.

University of California
Berkeley, CA
1972-1976

- Graduate research on the solution conformation of transfer RNA.
  Thesis title: Studies of Nucleic Acid Chemistry:
  Part I. The Solution Structure of Yeast Initiator Transfer RNA Studied by Oligonucleotide Binding
  Part II. A Chemical Model of Mutagenesis
  Experience in: isolation of tRNA, oligoribonucleotide synthesis, oligonucleotide hybridization, NMR spectroscopy.
  Research Advisor: Ignacio Tinoco Jr.

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Yogesh S. Sanghvi, Laurent Bellon, François Morvan, Tomonori Hoshiko, Eric Swayze, Lendell Cummins, Susan Freier, Nicholas Dean, Brett Monia, Richard H. Griffey and P. Dan Cook, "Synthesis, Biophysical, and Biological Evaluations of Novel Antisense Oligonucleosides Containing Dephosphono-internucelosidic Linkages", *Nucleosides & Nucleotides*, **14**, 1087-1090 (1995).

Elena A. Lesnik and Susan M. Freier, "Relative thermodynamic stability of DNA, RNA and DNA:RNA hybrid duplexes, relationship with base composition and structure", *Biochemistry*, **34**, 10807-10815 (1995).

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Susan M. Freier, Danielle A. M. Konings, Jacqueline R. Wyatt and David J. Ecker, "Mutational SURF: A Strategy for Improving Lead Compounds Identified from Combinatorial Libraries", *Bio-organic and Medicinal Chemistry*, **4**, 717-725 (1996).

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Brett Monia, Henri Sasmor, Joseph F. Johnston, Susan M. Freier, Elena A. Lesnik, Marcel Muller, Karl-Heinz Altmann, Heinz Moser and Doriano Fabbro, "Sequence-Specific Antitumor Activity of a Phosphorothioate Oligodeoxynucleotide Targeted to Human C-*raf* Kinase Supports and Antisense Mechanism of Action *in vivo*", *Proc. Natl. Acad. Sci. USA*, **93**, 15481-15484 (1996).

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Human tyrosine kinase, non-receptor, 1, ID: 12436, Cell Line: T-24, Oligo Conc: 150 nM, Primer Probe Set:RTS1356

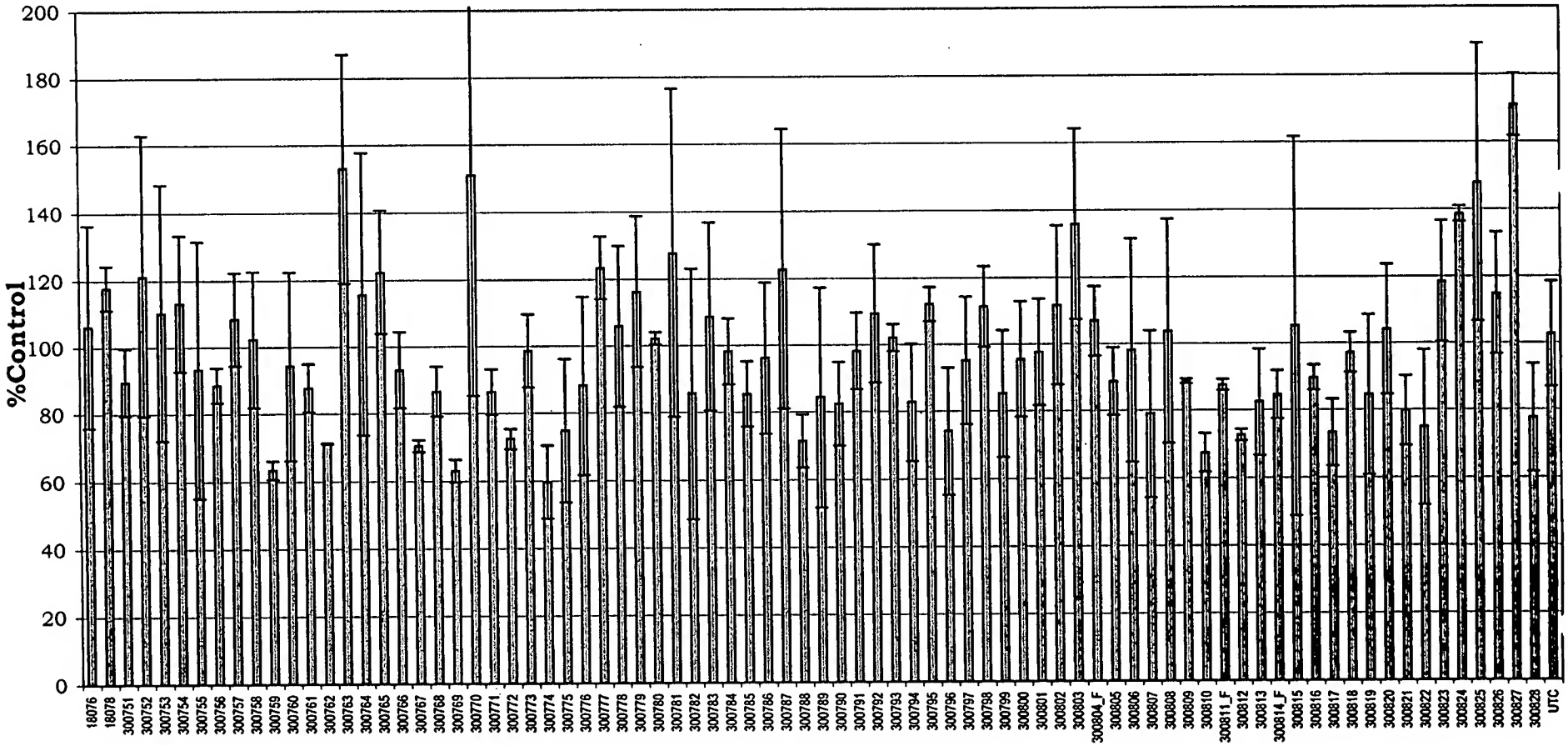
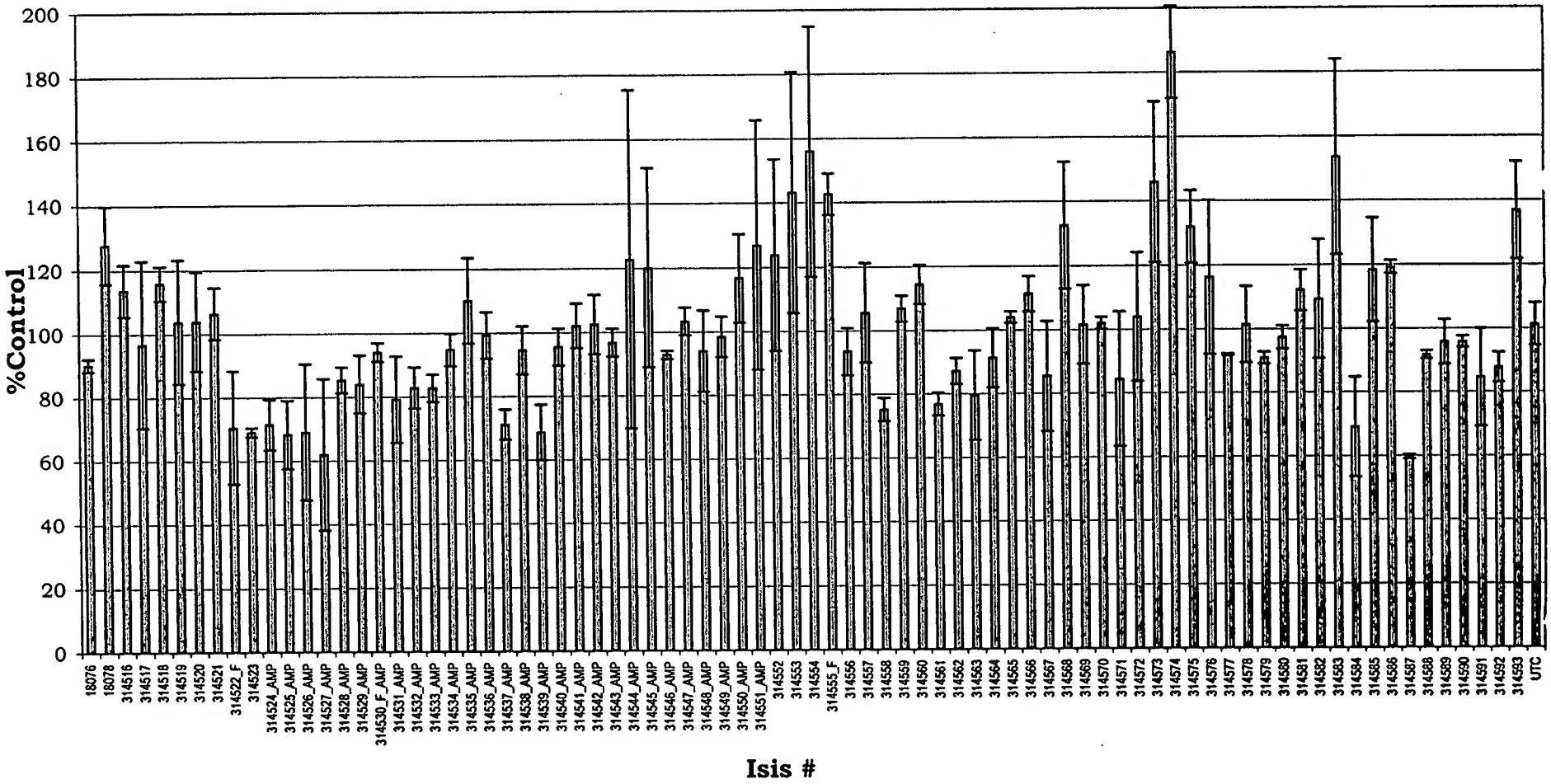


EXHIBIT 2

Rat urate anion exchanger 1, ID: 31543, Cell Line: Rin-M, Oligo Conc: 200 nM, Primer Probe Set:RTS1642



Isis #

EXHIBIT 3